

INHIBITION BY PYRUVATE OF PIG HEART MITOCHONDRIAL GLUTAMATE INFLUX

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Received 22 November 1977

1. Introduction

Cardiac muscle mainly oxidises fatty acids, ketone bodies and pyruvate originating from either glycolysis or glucogenolysis, or from lactate oxidation. The formation of pyruvate and its oxidation by mitochondria are controlled directly by the reoxidation of extra-mitochondrial NADH, which cannot operate directly via the respiratory chain [1,2]. This reoxidation probably occurs via the Borst cycle [3], which involves a glutamate-aspartate exchange in mitochondrial membrane [4]. As the redox potential of mitochondrial NAD is more electronegative than that of the extramitochondrial phase, it has been shown that the glutamate-aspartate exchange is an electrogenic process [5]. A protein with a high affinity for glutamate has been isolated from pig heart mitochondria [6].

In isolated heart mitochondria there is rapid, oxidation of glutamate catalysed by aspartate amino-transferase (EC 2.6.1.1). The addition of pyruvate has been shown to reduce considerably glutamate oxidation in heart mitochondria of rat [7], guinea pig [8] and pig [9]. This paper presents results indicating that glutamate translocation into pig heart mitochondria is strongly inhibited by pyruvate.

2. Material and methods

Pig heart mitochondria were isolated according to [10].

Abbreviations: Tris, Tris(hydroxymethyl)-aminomethane; MES, 2(*N*-morpholino) ethane sulfonic acid; Glu, Glutamate

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Mitochondrial protein was determined by the biuret method [11].

For the determination of the kinetics of glutamate incorporation, mitochondria (2–3 mg protein/ml) were suspended in a medium containing 0.2 M sucrose, 0.02 M MES-Tris pH 6.5 and [U-¹⁴C]sucrose by which the extramatrix space can be determined.

L-[³H]Glutamate was added and mitochondria were separated from the incubation medium after a given time by rapid centrifugation in an Eppendorf 3200 microcentrifuge.

The supernatant was discarded and the pellet quickly rinsed with 0.2 M sucrose and dissolved in 0.2 ml formic acid. The radioactivity of the formic acid extracts was measured in an Inter technique SL 40 scintillation counter in the POPOP/PPO system.

The amount of glutamate taken up by the mitochondria was calculated by correcting the total [³H]-glutamate in the mitochondrial pellet for the glutamate contained in the extramatrix space (3.5–4 μ l/mg protein according to the experimental conditions).

L-[G-³H]Glutamate and [U-¹⁴C]sucrose were obtained from the Radiochemical Centre, Amersham, Bucks.

3. Results and discussion

3.1. Kinetic parameters of glutamate influx

Glutamate penetration measured in state 3 follows a saturation curve shown in fig. 1. The K_m is 0.3–0.4 mM (20°C, pH 6.5) and V_{max} 6 nmol glutamate/min/mg protein. Between different mitochondrial preparations the state 3 V_{max} varies from 4.9–9.5 nmol/min/mg protein (for 16 expt. V_{max} 6.6 ± 0.5 nmol/min/mg protein). Comparable variation was observed in rat

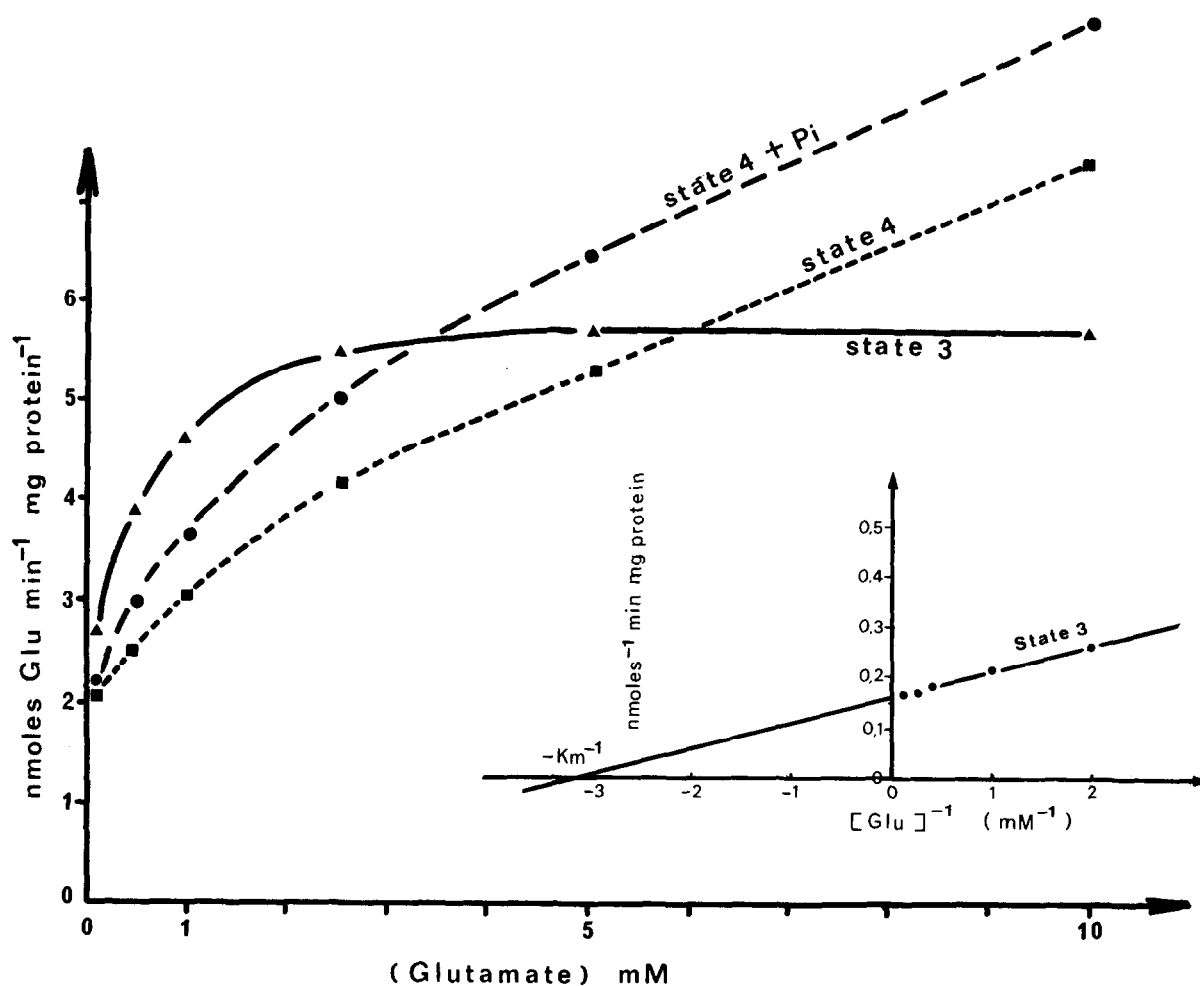


Fig.1. Concentration dependence of glutamate uptake. Mitochondria (2.5 mg protein) were added to 0.95 ml medium containing 200 mM sucrose, 20 mM MES-Tris, $[U-^{14}C]$ sucrose, glutamate concentration was varied from 0.1–10 mM. State 3 was obtained by addition of 0.1 mM ADP and 2.5 mM P_i . pH 6.5, 20°C and incubation time 1 min.

liver mitochondria with V_{max} values of 23 nmol [12] and 9.1 nmol/min/mg protein [13] being measured under very similar conditions. Affinity appears to be reduced when the measurements are made in state 4, and saturation no longer occurs. The rates of glutamate penetration measured in the presence of rotenone are similar to those measured in state 4 (results not given here). P_i , 2.5 mM, added in state 4 increases glutamate uptake and this effect continues in the presence of mersalyl, which inhibits P_i transport (results not given here). P_i was observed to increase aspartate efflux from rat heart mitochondria [14].

3.2. Inhibition by pyruvate of glutamate influx

Figure 2 shows that pyruvate is a powerful inhibitor of glutamate entry.

Plots of $1/V$ against pyruvate concentration, at different glutamate concentrations, are biphasic (fig.3). For 0.1–1 mM pyruvate inhibition is competitive and the K_i is 0.6–0.7 mM. Below 0.1 mM the affinity of pyruvate for the transport system is increased. These results suggest that there is a physiological regulation mechanism between pyruvate concentration and inhibition of glutamate translocation linked to the reoxidation of extramitochondrial NADH.

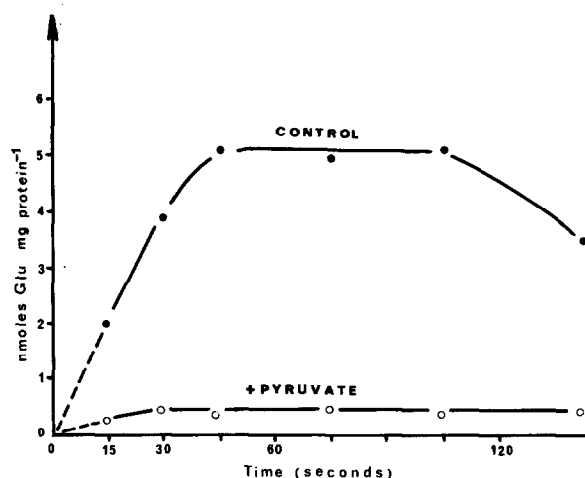


Fig.2. Time course of L-[^3H]glutamate penetration in state 4 and inhibition by pyruvate. Same conditions as in fig.1, except 1 mM glutamate. Pyruvate, 2 mM, was added 10 s before glutamate.

Figure 4 shows that pyruvate is not the only inhibitor of glutamate uptake, α -ketoglutarate or oxaloacetate are also effective, although less so than pyruvate, and structural analogues such as α -ketobutyrate or phenylpyruvate also inhibit. In contrast, malate has no inhibiting effect. One may note that all the inhibitory molecules obtain the $-\text{CO}-\text{COOH}$ group.

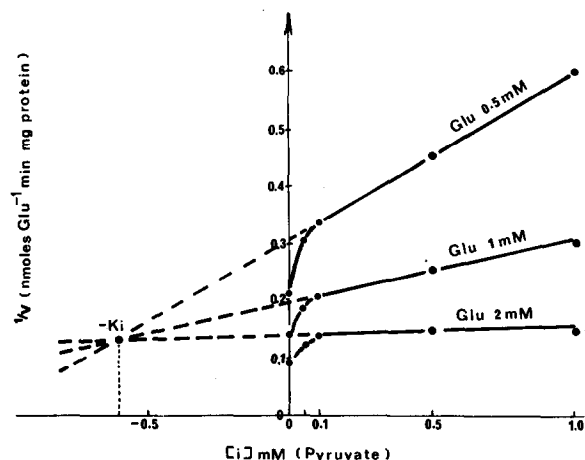


Fig.3. Effect of pyruvate concentration on L-[^3H]glutamate uptake, determination of K_i for pyruvate in state 4. Same conditions as in fig.1. Pyruvate concentration was varied from 0–1 mM.

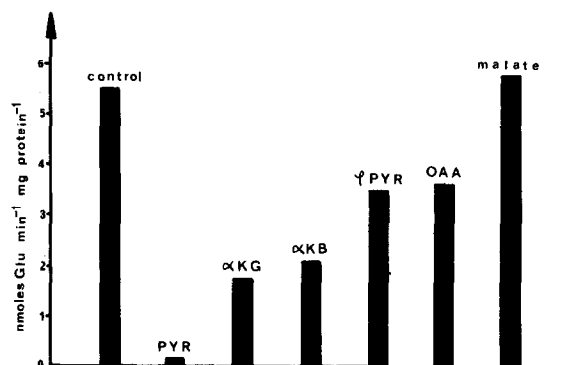


Fig.4. Inhibition of glutamate uptake by pyruvate and analogues in state 3. 1 mM glutamate; 5 mM pyruvate (Pyr), α -ketoglutarate (αKG), α -ketobutyrate (αKB), phenylpyruvate (ϕPyr), oxaloacetate (OAA), or malate were added 10 s before glutamate.

As a first hypothesis, one can suggest that pyruvate and its structural analogues act on the transport system which controls glutamate entry into mitochondria. A second hypothesis is that the effect of substrates with the $-\text{CO}-\text{COOH}$ group (pyruvate, α -ketoglutarate and oxaloacetate) could be due to their intramitochondrial metabolism (fig.5). The inhibitory effect of α -ketoglutarate could result from a reduction in aspartate amino-transferase activity following uptake of this substrate. In contrast, oxalo-

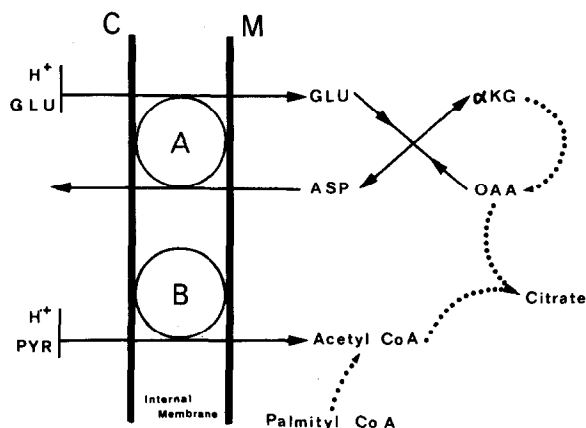


Fig.5. Translocation of glutamate and pyruvate in pig heart mitochondria. (A) Electrogenic exchange glutamate-aspartate according to [5]. (B) Pyruvate carrier—pyruvate dehydrogenase complex. (C) Cytosolic face. (M) Matrix face.

acetate, which can diffuse easily in heart mitochondria [15,16], should stimulate glutamate entry, whereas, as the result in fig.4 shows, it causes inhibition.

Malate, which can give rise to oxaloacetate in mitochondria has no inhibitory effect on glutamate translocation, suggesting that intramitochondrial oxaloacetate does not inhibit glutamate translocation while exogenous oxaloacetate does (fig.5).

3.3. Effects of rotenone, arsenite and Ca^{2+} on glutamate influx, in the absence and presence of pyruvate

Following the second hypothesis, the inhibition of glutamate entry by pyruvate could be explained by competition for oxaloacetate between aspartate amino transferase (EC 2.6.1.1) and citrate synthase (EC 4.1.3.7.) (fig.5). In these conditions inhibition or activation of pyruvate dehydrogenase would affect glutamate translocation.

Figure 6 (exp. a) shows that rotenone reduces by about 15% glutamate translocation measured in state 3. Addition of rotenone slightly reduces the inhibition

of glutamate translocation by pyruvate (1 nmol glutamate/mg protein/min in the presence of pyruvate + rotenone instead of 0.2 nmol in the presence of pyruvate alone). Rotenone, which inhibits oxidative decarboxylation of pyruvate thus has little effect on glutamate translocation in the presence of pyruvate.

Sodium arsenite, a powerful inhibitor of pyruvate dehydrogenase, markedly reduces inhibition of glutamate translocation (fig.6, exp. b).

The addition of 0.2 mM Ca^{2+} (fig.6, exp. c) has little effect on glutamate translocation. In contrast, Ca^{2+} , an activator of phosphatase linked to the action of pyruvate dehydrogenase, significantly reinforces the inhibitory effect of pyruvate.

3.4. Effects of palmityl CoA oxidation and amino-oxycetate on glutamate influx

The results in fig.7 show that palmityl CoA oxidation (a generator of intramitochondrial acetyl CoA) does not affect influx, while amino-oxycetate, an aspartate amino-transferase inhibitor [18], reduces glutamate translocation by only 30%.

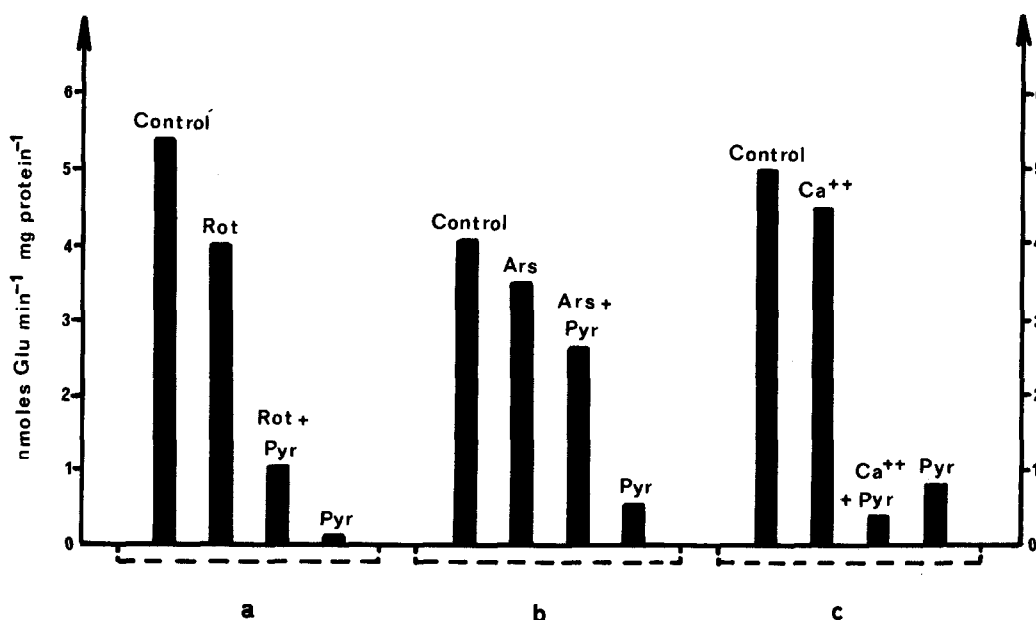


Fig.6. Effect of rotenone, sodium-arsenite and calcium on L-[³H]glutamate uptake in presence of pyruvate in state 3. Same conditions as in fig.1 for the three experiments. (a) Effect of rotenone (Rot) (15 $\mu\text{g}/\text{mg}$ protein). (b) Effect of 1 mM sodium-arsenite (Ars). (c) Effect of 0.2 mM calcium. Pyruvate, 5 mM, was added 10 s before glutamate and rotenone, sodium arsenite or calcium 2 min before pyruvate.

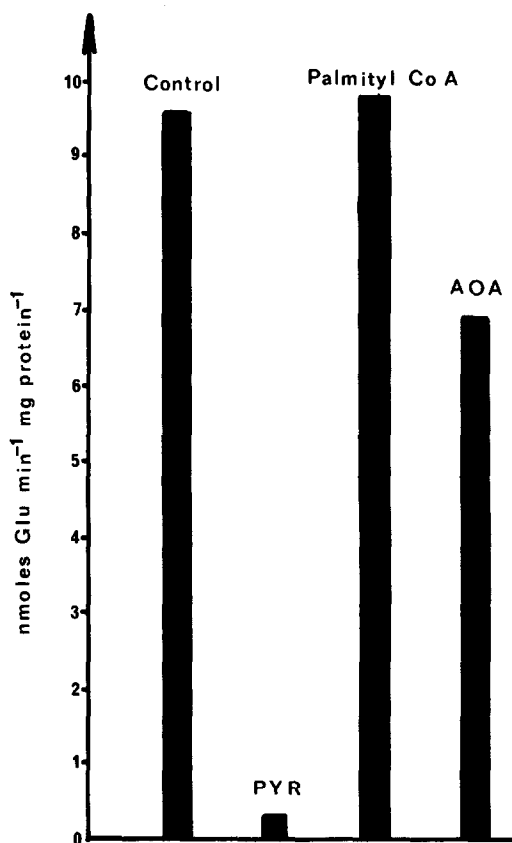


Fig.7. Effect of palmityl CoA oxidation and aspartate aminotransferase inhibition by amino-oxycetate on L-[³H]glutamate uptake. Same conditions as in fig.1 except: state 3 was obtained by addition of 2 mM ADP and 2.5 mM P_i. Pyruvate, 5 mM, was added 10 s before glutamate. Carnitine, 5 mM, + palmityl CoA, 5 μ M, or 10 mM amino-oxycetate (AOA) were added 2 min before glutamate.

4. Conclusions

The inhibition of glutamate oxidation in heart mitochondria by pyruvate follows inhibition of glutamate transport by this substrate. Several hypotheses could explain this inhibition:

1. Pyruvate is a competitive inhibitor which acts directly on the glutamate binding site of the transport system. All structural analogues studied containing the -CO-COOH group are inhibitors.
2. Inhibition of glutamate translocation follows competition for intramitochondrial oxaloacetate. Only

the results obtained in the presence of Na arsenite support this hypothesis.

3. One of us (Stepien, G., unpublished) has shown that pyruvate translocation in pig heart mitochondria is controlled by the transmembrane Δ pH. It is possible that in the membrane there is competition for energy between glutamate transport and pyruvate transport.

Acknowledgement

Thanks are due to the DGRST (Convention 76.7.II71).

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